#36

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re :	U. S. Patent No. 4,978,655
Issued:	December 18, 1990
To :	Tai-Shun Lin and William Prusoff
For :	Use of 3'-Deoxythymidin-2'-ene(3'deoxy-2',3'-didehydrothymidine) in treating patients infected with retroviruses

Commissioner of Patents and Trademarks
Box Patent Extension
Washington, D. C. 20231

APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. 156

Dear Sir:

Your Applicant, Yale University, a corporation organized and existing under and by virtue of a charter granted by the general assembly of the Colony and State of Connecticut, United States of America and having its principal office at 451 College Street, New Haven, Connecticut 06520 represents that it is the assignee of the entire interest in and to Letters Patent of the United States Patent No. 4,978,655 ('655 Patent) granted to Tai-Shun Lin and William Prosoff on the 18th day of December 1990 for Use of 3'-Deoxythymidin-2'-ene(3'deoxy-2',3'-didehydrothymidine) in treating patients infected with retroviruses. Your Applicant, acting through its duly authorized Agent, Bristol-Myers Squibb Company, a corporation of the State of Delaware and the undersigned attorney, hereby submits this application

for extension of patent term under 35 U.S.C. 156 by providing the following information required by the guidelines and rules published by the U. S. Patent and Trademark Office. An originally executed Authorization of Agent and Power of Attorney evidencing the appointment of Bristol-Myers Squibb Company and the undersigned as duly appointed Agent is attached hereto as "Exhibit 1".

As a result of an Agreement dated December 23, 1987 with Yale University, Bristol-Myers Squibb Company is the Licensee of the exclusive rights to the '655 patent, having the exclusive license to make, use and sell the compound 1-(2,3-dideoxy-B-D-glycero-pent-2-enofuranosyl) thymidine, an antiretroviral (HIV) nucleoside also known as stavudine, said anti-HIV use being claimed in said patent, throughout the entire United States for the full and true term of the '655 patent. In conjunction with its exclusive license under the '655 patent, Bristol-Myers Squibb Company, at a cost of many millions of dollars, has undertaken through its United States Pharmaceutical Division, the commercial development of stavudine over the past several years including the performance of extensive clinical trials pursuant to an Investigational New Drug Application ("IND") and the ultimate filing of a New Drug Application ("NDA") in order to obtain approval by the U.S. Food and Drug Administration ("FDA") for the commercial marketing of stavudine. On June 24, 1994, the FDA granted approval of Bristol-Myers Squibb's NDA on the use of a composition containing stavudine as an anti-HIV agent. This FDA approval constituted the first permitted commercial marketing or use of stavudine.

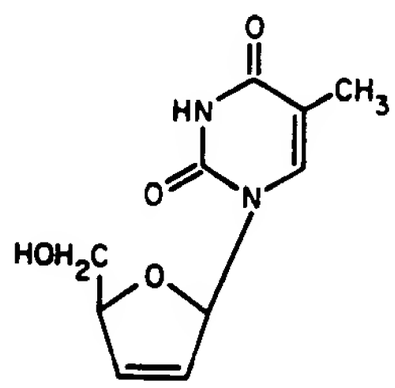
Based on these IND and NDA regulatory review periods and in accordance with the provisions of 35 U.S.C. §156, Yale University with the approval and consent of Bristol-Myers Squibb Company, the holder of the regulatory approval granted with respect to the IND and NDA regulatory review periods, is hereby seeking the requested extension of the '655 patent.

SECTION 1

Complete Identification of the Approved Product.

The approved product, which is known generically as stavudine and which will be marketed by the Pharmaceutical Division of Bristol-Myers Squibb Company under the tradename **ZERIT**, has:

a) The structural formula



b) The empirical formula, $C_{10}H_{12}N_2O_4$

c) A molecular weight of 224.22

d) The chemical name: 1(2,3-dideoxy-ß-D-glycero-pent-2-enofuranosyl)thymidine

SECTION 2

Complete identification of the Federal statute including the applicable provision of law under which the regulatory review occurred.

Pursuant to Section 505 (c) (1) (A) of the Federal Food, Drug and Cosmetic Act [21 U.S.C. 355 (c) (1) (A)] NDA #20-412 was approved and received permission for commercial marketing on June 24, 1994. The regulatory review period began on March 1, 1989 relative to the medical indication of **ZERIT** as an anti-HIV agent, approximately 75 days after receipt by the FDA of IND #32486. A chronology of the regulatory review period is provided in Sections 11 and 12.

SECTION 3

Identification of the date on which the product received permission for commercial marketing.

NDA #20-412 was approved on June 24, 1994 pursuant to Section 505 (c) of the Federal Food, Drug and Cosmetic Act.

SECTION 4

Identification of each active ingredient in the product.

Stavudine is the only active ingredient of the approved product and has not been previously approved for commercial marketing or use.

SECTION 5

This application is being submitted within the sixty (60) day period pursuant to 37 CFR 1.720 (f) since NDA approval was granted on June 24, 1994 and the sixty day period will lapse on August 23, 1994.

SECTION 6

Complete identification of the patent.

The patent for which an extension is sought is U.S. Patent No. 4,978,655 which was issued on December 18, 1990 and set to expire on December 18, 2007. The patent is based on U.S. Patent Application No. 942,666 filed by Tai-Shun Lin and William Prusoff on December 17, 1986.

SECTION 7

A copy of U.S. Patent No. 4,978,655 which is entitled Use of 3'-Deoxythymidin-2'-ene(3'deoxy-2',3'-didehydrothymidine) in treating patients infected with retroviruses is provided as "Exhibit 2".

SECTION 8

A copy of any disclaimer, certificate of correction, receipt of maintenance fee payment or reexamination certificate issued in the patent.

A copy of a certificate of correction dated October 27, 1992 is provided as "Exhibit 3".

(Section 9 begins on the next page)

SECTION 9

Statement that the patent claims the approved product.

U.S. Patent No. 4,978,655 claims the use of the compound "stavudine" (which is the active ingredient in the approved product) in treating HIV infections.

Claim 1 of U.S. Patent No. 4,978,655 covers the use of the compound "stavudine" in treating retroviral infections.

Claim 8 of U.S. Patent No. 4,978,655 covers the specific use of the compound "stavudine" in treating HIV infections.

(Section 10 begins on the next page)

SECTION 10

Relevant dates and information pursuant to 35 U.S.C. 156 (g) to enable a determination of the applicable regulatory review period.

March 1, 1989	Effective date of Notice of claimed Investigational Exemption (IND) for use of **ZERIT** in the treatment of HIV infection (IND No. 32486)
December 28, 1993	Date of receipt of New Drug Application (NDA) No. 20-412 for **ZERIT** by the U.S. FDA
June 24, 1994	NDA approval date

(Section 11 begins on the next page)

SECTION 11

Brief description of the activities undertaken by the applicant during the applicable regulatory review period with respect to the approved product and significant dates applicable to such activities.

The Bristol-Myers Squibb Company which through an Agreement dated December 23, 1987 is the exclusive licensee of the compound stavudine, undertook the development of **ZERIT** to establish by adequate and well-controlled clinical trials, its safety and effectiveness as an agent for the tretment of HIV infection. Since the product was a new drug as defined under Section 201 (P) of the Federal Food, Drug and Cosmetic Act, an approved NDA for the product was required to be obtained under Section 505 (b) of said Act prior to its commercial marketing.

A Notice of Claimed Investigational Exemption for a New Drug (IND) was submitted on December 15, 1988 and received by FDA on December 16, 1988 for an oral formulation of **ZERIT** under subsection (1) of Section 505 of the Federal Food, Drug and Cosmetic Act. The notice provided for the clinical evaluation of **ZERIT** as an agent to treat HIV infection. The FDA did request that the sponsor, The U. S. Pharmaceutical Group of Bristol-Myers Squibb Company withhold the use of **ZERIT** in human subjects until March 1, 1989 after the above submission. The chronological information listed below refers to information or studies relating to the development of this indication.

The following is a chronology of the activity that ensued:

December 15, 1988	IND submitted to FDA
December 16, 1988	IND #32486 received by FDA
MARCH 1, 1989	FDA Letter confirming permission to proceed with clinical trials i.e. lifting the clinical hold.
April 5, 1989	Letter to FDA. "Summary of dosing experience and demographic information."
April 27, 1989	Letter to FDA. "Request meeting to discuss preclinical and clinical development plans."
July 10, 1989	Phone call to FDA. "Summary of dosing and ADE's and request for escalation to next dose level and extended duration of dosing to one year approved."
August 1, 1989	Submission of Four-Week Oral Toxicity Study in Cynomolgus Monkeys with stavudine.
November 11, 1989	Submission of Method for the Quantitative Analysis of stavudine in Human Urine by High Pressure Liquid Chromatography.
December 15, 1989	Submission of Acute Toxicity of stavudine in Cynomolgus Monkeys by Intravenous Administration.
April 18, 1990	A Dose Ranging Safety, Pharmacokinetic and Preliminary Efficacy Study of stavudine (2'3'-didehydro-3'deoxythymidine d4T) Administered Three Times Daily to symptomatic HIV-Infected Patients.
July 25, 1990	Submission of A Segment II Teratology Study in Rabbits with stavudine.
November 9, 1990	Submission of Central Nervous System Pharmacodynamic Safety Studies in Rats of stavudine.
November 9, 1990	Submission of A Cardiovascular System Pharmacodynamic Safety Study in the Rats of stavudine.
DECEMBER 18, 1990	U. S. Patent No. 4,978,655 issued.

March 14, 1991	Submission of A Phase I Safety and Pharmacokinetic/Pharmacodynamic Analysis of stavudine (2'3'-didehydro-3'deoxythymidine d4T) Administered Three Times Daily to Patients with HIV Infection. Original Submission and Incorporates Amendment No. 1.
June 26, 1991	Submission of Acute Toxicity of stavudine in Neonatal, Weanling and Adult Rats by Oral Administration.
August 6, 1991	Submission of A Segment II Teratology Study in the Rat with stavudine.
September 6, 1991	Submission of a Combined Analysis of the Phase I/II Studies of stavudine.
September 6, 1991	Submission of an Overview of the Animal Toxicology Studies with stavudine.
September 6, 1991	Submission of a Summary of the Biopharmaceutics of stavudine.
November 13, 1991	Submission of a Phase I Safety, Pharmacokinetic and Efficacy Study of stavudine Administered Orally Twice Daily to children with HIV Infection.
March 12, 1992	Submission of a Double-Blind Comparison of ZDV and stavudine for Treatment of Patients with HIV Infection Who Have Received at Least Six Months of ZDV Therapy and Who Have Absolute CD4 Lymphocyte Counts Between 50 and 500 Cells/mm^3.
July 17, 1992	Submission of a Randomized Blinded Evaluation of Two Doses of Stavudine to Make Treatment Available in Severely Immuno-Compromised Patients with HIV Infection Who Have Failed or are Intolerant of Alternative Anti-Retroviral Therapy.
September 6, 1992	Interim Analysis: Population Pharmacokinetics of stavudine in Patients with AIDS or ARC.
December 16, 1992	Two Year Dietary Carcinogenicity Study in Mice.

January 29, 1993	Submission of a Chronic (12 months) Oral Toxicity Study in Rats.
April 20, 1993	Submission of A Pilot Study of stavudine for the Treatment of Wasting in Patients with Symptomatic HIV Infection.
May 24, 1993	Submission of a Pilot Randomized Double-Blind Study to Compare the Safety and Biological Effects of Combinations of Didanosine and Stavudine in HIV-Infected Subjects with CD4 Cell Counts of 200-500/μL and with No Prior Antiretroviral Therapy.
July 6, 1993	Submission of Pharmacokinetics and Dose-Proportionality Study of Stavudine.
DECEMBER 28, 1994	NDA submitted and received at FDA.
January 28, 1994	Post-NDA. Meeting at FDA regarding clinical and preclinical/technical sections of the NDA.
February 10, 1994	Revised labeling.
February 15, 1994	Response to FDA questions re: statistical information.
February 28, 1994	Submission of a Single-Dose Safety and Pharmacokinetic Study of stavudine in Subjects with Hepatic Impairment.
March 11, 1994	Interim study report for Protocol AI 455-010.
March 23, 1994	Letter regarding the four (4) month safety update.
April 7, 1994	DSMB Safety and Efficacy Report
April 13, 1994	CMC Section amendment.
May 4, 1994	120-day safety update.
May 20, 1994	Antiviral Drugs Advisory Committee Agenda for May 20 Meeting.
June 2, 1994	Revised Draft Labeling
June 15, 1994	Phase IV Commitments letter.
JUNE 24, 1994	NDA #20-412 approved by FDA.

SECTION 12

Patent extension eligibility and the length of extension claimed.

The '655 patent is eligible for an extension of 188 days based on the following:

(a) The '655 patent specifically claims the compound "stavudine" which is the active ingredient of the approved human drug product, **ZERIT**;

(b) The term of the '655 patent has never been previously extended;

(c) This Application for Patent Term Extension of the '655 patent is submitted by Yale University in compliance with 37 C.F.R §1.740;

(d) The approved product **ZERIT** has been subject to a regulatory review period as defined in 35 U.S.C. §156 (g) prior to its commercial marketing or use;

(e) The approved product received permission for commercial marketing or use on June 24, 1994 and this permission is the first received permission for commercial marketing or use of the approved product under the provision of law under which the applicable regulatory review occurred;

(f) This Application for Patent Term Extension of the '655 patent is submitted within the sixty day period beginning on June 24, 1994 the date that the approved product first received permission for commercial marketing or use under the provision of law under which the applicable regulatory review period

occurred;

(g) The term of the '655 patent has not expired prior to the submission of this application; and

(h) No other patent term has been extended for the same regulatory review period for this approved product.

The length of the patent term extension claimed is the less of 5 years or the following calculated period of time:

Patent issued:	**December 18, 1990**
Testing period began:	**March 1, 1989**
NDA submitted:	**December 28, 1993**
NDA approved:	**June 24, 1994**

The testing period would equal the time between March 1, 1989 and December 27, 1993, or 1763 days.

The approval period would equal the time between December 28, 1993 and June 24, 1994, or 179 days.

(1) The total extension would equal 1/2 x 1763 + 179 or 1060 days to the term of the patent expiration date i.e. December 18, 2007, which would extend the patent term to November 7, 2010.

(2) Alternatively, the addition of fourteen (14) years to the date of NDA approval i.e. June 24, 1994 would lead to a date of June 24, 2008.

Since the period of time computed in (2) above is less than that of (1) the extension requested is for a 188 day period.

SECTION 16

Duplicate Application Paper

A duplicate of this Application For Patent Term Extension of the '655 patent hereby certified as such, is being submitted herewith.

SECTION 17

Oath or declaration

The following Declaration of Dominic M. Mezzapelle is submitted herewith in compliance with the requirements of 37 C.F.R. Section 1.740 (b):

Declaration

The undersigned acting pursuant to an Authorization of Agent and Power of Attorney executed by Yale University, the applicant submitting this Application for Patent Term Extension of United State Patent No. 4,978,655 hereinabove referred to as the '655 patent, in compliance with the requirements of 37 C.F.R. §1.740 (b) (1), hereby avers as follows:

1. He is a patent attorney authorized to practice before the U.S. Patent and Trademark Office (Reg. No. 20,806) and pursuant

SECTION 13

Duty of Disclosure

The applicant, Yale University hereby acknowledges its duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to the determination of entitlement to this application for patent term extension.

SECTION 14

Prescribed Fee

The prescribed fee ($1,000.00) for receiving and acting upon this Application For Patent Term Extension of the '655 patent is to be charged to Deposit Account No. 02-3850.

SECTION 15

Inquiries and Correspondence

Inquiries and correspondence relating to this Application For Patent Term Extension of the '655 patent should be directed to:

Dominic M. Mezzapelle
Associate General Counsel-Patents
Bristol-Myers Squibb Company
345 Park Avenue
New York, New York 10154
Telephone 212-546-3651

to an Authorization of Agent and Power of Attorney from Yale University, the assignee of record of the '655 patent, a copy of which is attached as Exhibit 1, he is authorized to represent Yale University in this Application for Patent Term Extension of the '655 patent and to transact all business in the United States Patent and Trademark Office in connection therewith:

2. He has reviewed and understands the contents of this Application for Patent Term Extension of the '655 patent;

3. He believe that the '655 patent is subject to patent term extension pursuant to the provision of 37 C.F.R. §1.710;

4. He believes that the extension of the length claimed in this Application for Patent Term Extension of the '655 patent is justified under 35 U.S.C. §156 and the applicable regulations relating thereto; and

5. He believes that the '655 patent which is the subject of this Application for Patent Term Extension meets the conditions for patent term extension as set forth in 37 C.F.R. §1.720.

Respectfully submitted,

YALE UNIVERSITY

By 

Dominic M. Mezzapelle
Reg. No. 20,806

DATE: AUGUST 18, 1994

all being employees of Bristol-Myers Squibb Company, individually and collectively to be the agents and attorneys of Yale University with regard to an application for extension of the term of U. S. Patent No. 4,978,655 and to transact all business in the U. S. patent and Trademark Office in connection therewith.

Please address all communications in the above matter to:

Dominic M. Mezzapelle
Associate General Counsel - Patents
Bristol-Myers Squibb Company
345 Park Avenue
New York, New York 10154

YALE UNIVERSITY

BY: _____

NAME: Linda K. Lorimer

TITLE: Secretary

DATE: August 17, 1994

EXHIBIT 1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re:	U. S. Patent No. 4,978,655
Issued:	December 18, 1990
To:	Tai-Shun Lin and William H. Prusoff
For:	Use of 3'-Deoxythmidin-2-ene(3'deoxy-2'.3'-didehydrothmidine) in treating patients infected with retroviruses

Commissioner of Patents and Trademarks
Box Patent Extension
Washington, D. C. 20231

AUTHORIZATION OF AGENT AND
POWER OF ATTORNEY

Yale University, a corporation organized and existing under and by virtue of a charter granted by the assembly of the Colony and the State of Connecticut and having its principal office at 451 College Street, New Haven, Connecticut, 06520 being the owner of record of the above identified U. S. Letters Patent, hereby authorize and appoint, Bristol-Myers Squibb Company, a corporation organized and existing under the laws of the State of Delaware and having its principal office at 345 Park Avenue, New York, New York 10154, and the Patent Attorneys named below:

Dominic M. Mezzapelle	**(Reg. No. 20,806)**
Donald J. Barrack	**(Reg. No. 26,414)**
Frank P. Hoffman	**(Reg. No. 26,468)**
David M. Morse	**(Reg. No. 25,742)**

EXHIBIT 2

United States Patent [19]

Lin et al.

[54] **USE OF 3'-DEOXYTHYMIDIN-2'-ENE (3'DEOXY-2',3'-DIDEHYDROTHYMIDINE) IN TREATING PATIENTS INFECTED WITH RETROVIRUSES**

[75] Inventors: **Tai-Shun Lin**, North Haven; **William H. Prusoff**, North Branford, both of Conn.

[73] Assignee: **Yale University**, New Haven, Conn.

[21] Appl. No.: **942,666**

[22] Filed: **Dec. 17, 1986**

[51] **Int. Cl.**[5] **A61K 31/70**
[52] **U.S. Cl.** **514/50**; 514/934
[58] **Field of Search** 536/23; 514/49; 574/50

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[45] **Date of Patent: Dec. 18, 1990**

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(List continued on next page.)

Primary Examiner—John W. Rollins
Attorney, Agent, or Firm—Sprung Horn Kramer & Woods

[57] **ABSTRACT**

This invention relates to the use of 3'-deoxythymidin-2'-ene (3'deoxy-2',3'-didehydrothymidine) in treating patients infected with a retrovirus.

8 Claims, No Drawings

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Derwent abstract No. 88-170966/25.

USE OF 3'-DEOXYTHYMIDIN-2'-ENE (3'DEOXY-2',3'-DIDEHYDROTHYMIDINE) IN TREATING PATIENTS INFECTED WITH RETROVIRUSES

GOVERNMENT RIGHTS

This invention was made with United States government support under Grant CA-28852 from the NIH. The United States Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention concerns the use of 3'-deoxythymidin-2'-ene (3'-deoxy-2', 3 -didehydrothymidine) in treating patients infected with retroviruses.

2. Background Information

Acquired immunodeficiency syndrome (AIDS) is generally accepted to be a consequence of infection with the retrovirus variously termed human T-lymphotropic virus type III (HTLV-III), lymphadenopathy-associated virus (LAV), AIDS associated retrovirus (ARV), or human immunodeficiency virus (HIV). A number of compounds have demonstrated antiviral activity against this virus which include HPA-23 (D. Dormont, B. Spire, F. Barre-Sinoussi, L. Montagnier and J. C. Chermann, *Ann. Inst. Pasteur/Virol.*, 75, 136E, (1985) and W. Rosenbaum, D. Dormont, B. Spire, E. Vilmer, M. Gentilini, C. Griscelli, L. Montagnier, F. Barre-Sinoussi and J. C. Chermann, *Lancet* i, 450, (1985)), interferons (D. D. Ho, K. L. Hartshorn, T. R. Rota, C. A. Andrews, J. C. Kaplan, R. T. Schoolkey and M. S. Hirsch, *Lancet*, i, 602, (1985)), , ribavirin (J. B. McCormick, J. P. Getchell, S. W. Mitchell and D. R. Hicks, *Lancet*, ii, 1367, (1984)), phosphonoformate (E. G. Sandstrom, J. C. Kaplan, R. E. Byington and M. S. Hirsch, *Lancet*, i 1480 (1984) and P. S. Sarin, Y. Taguchi, D. Sun, A. Thornton, R. C. Gallo and B. Oberg, *Biochem. Pharmac.*, 34, 4075, (1985)), ansamycin (R. Anand, J. L. Moore, A. Srinivason, V. Kalyanaraman, D. Francis, P. Feorino and J. Curran, *Abstracts of the International Conference on Acquired Immune Deficiency Syndrome (AIDS)*, April 14–17, Atlanta, GA, page 72, (1985)), suramin (H. Mitsuya, M. Popovic, R. Yarchoan, S. Matsushita, R. C. Gallo and S. Broder, *Science*, 226, 172, (1984); H. Mitsuya, S. Matsushita, M. E. Harper and S. Broder, *Cancer Res.*, 45, 4583s, (1985) and E. DeClercq, *Cancer Lett.*, 8, 9, (1979)), imuthiol (A. Pompidou, D. Zagury, R. C. Gallo, D. Sun. A. Thornton and P. S. Sarin, *Lancet*, ii, 1423, (1985)), penicillamine (P. Chandra and P. S. Sarin, *Drug Res.*, 36, 184, (1986)), rifabutin (R. Anand, J. Moore, P. Feorino, J. Curran and A Srinivasan, *Lancet*, i, 97, (1986)), AL-721 (P. S. Sarin, R. C. Gallo, D. I. Scheer, F. Crews and A. S. Lippa, *New Engl. J. Med.*, 313, 1289, (1985)), 3'-azido-3'deoxythymidine (W. Ostertag T. Cole, T. Crozier, G. Gaedicke, J. Kind, N. Kluge, J. C. Krieg. G. Roselser, G. Sheinheider, B. J. Weimann and S. K. Dube, *Proceedings of the 4th* International Symposium of the Princess Takamatsu Cancer Research Fund, Tokyo, 1973, *Differentiation and Control of Malignancy of Tumor Cells*. Eds. W. Nakahara, T. Ono, T. Sugimura and H. Sugano, page 485, University of Tokyo Press, Tokyo, (1974); W. Ostertag, G. Roseler, C. J. Kreig, T. Cole, T. Crozier, G. Gaedicke, G. Steinheider, N. Kluge and S. K. Dube, *Proc. Natn. Acad. Sci. USA*, 71, 4980, (1974); S. L. Dube, G. Gaedicke, N Kluge, B. J.

Weimann, H. Melderis, G. Steinheider T. Crozier, H. Beckmann and W. Ostertag, *Proceedings of the 4th* International Symposium of the Princess Takamatsu Cancer Research Fund, Tokyo, 1973, *Differentiation and Control of Malignancy of Tumor Cells*, Eds. W. Nakahara, T. Ono, T. Sugimura and H. Sugano, page 99, University of Tokyo Press, Tokyo, (1974); S. K. Dube, I. B. Pragnell, N. Kluge, G. Gacdicke, G. Steinheider and W. Ostertag, *Proc. Natn. Acad. Sci. USA*, 72, 1863, (1975) and H. Mitsuya, K. J. Weinhold, P. A. Furman, M. H. St. Clair, S. Lehrman Nusinoff, R. C. Gallo, D. Bolognesi, D. W. Barry and S. Broder, *Proc. Natn. Acad. Sci. USA*, 82, 7096, (1985)), and more recently various 2', 3'-dideoxynucleosides (H. Mitsuya and S. Broder, *Proc. Natn. Acad. Sci. USA*, 83, 1911, (1986)), of which 2',3'-dideoxycytidine (ddCyd) is the most potent. A review of these and other compounds evaluated for their activities against HIV, as well as a discussion of the AIDS problem in general, has been presented (E. DeClercq, *J. Med. Chem.*, 29, 1561, (1986)).

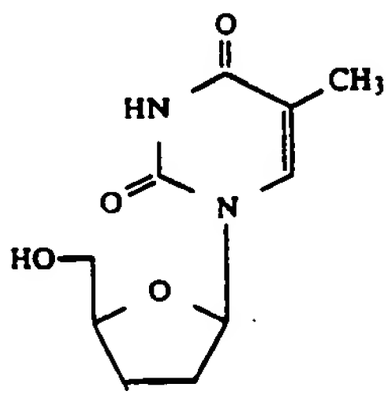
Applicants previously found 2', 3'-dideoxycytidin-2'-ene (2', 3'-dideoxy-2', 3'-didehydrocytidine; D4C) a derivative of 2', 3'-dideoxycytidine (ddCyd) to have antiviral activity against HIV (Lin et al, *Biochem. Pharmacol*, in press). This provided the stimulus to synthesis 3'-deoxythymidin-2'-ene (3'-deoxy-2', 3'-didehydrothymidine) even though Mitsuya and Broder, supra found 2', 3'-dideoxythymidine (3'-deoxythymidine) to be a very poor inhibitor of HTLV-III/LAV. Applicants' finding of potent antiviral activity with 3'-deoxythymidin-2'-ene was, therefore, unexpected based on their report.

SUMMARY OF THE INVENTION

The present invention is directed to the treatment of warm blooded animals, including humans, infected with a retrovirus comprising administering to a warm blood animal, e.g., a human patient, an anti-retroviral effective amount of 3'-deoxythymidin-2'-ene (3'-deoxy-2', 3'-didehydrothymidine), either alone or in admixture with a diluent or in the form of a medicament.

DETAILED DESCRIPTION OF THE INVENTION

The structure of 3'-deoxythymidin-2'-ene (3'-deoxy-2', 3'-didehydrothymidine; D4T) is as follows:

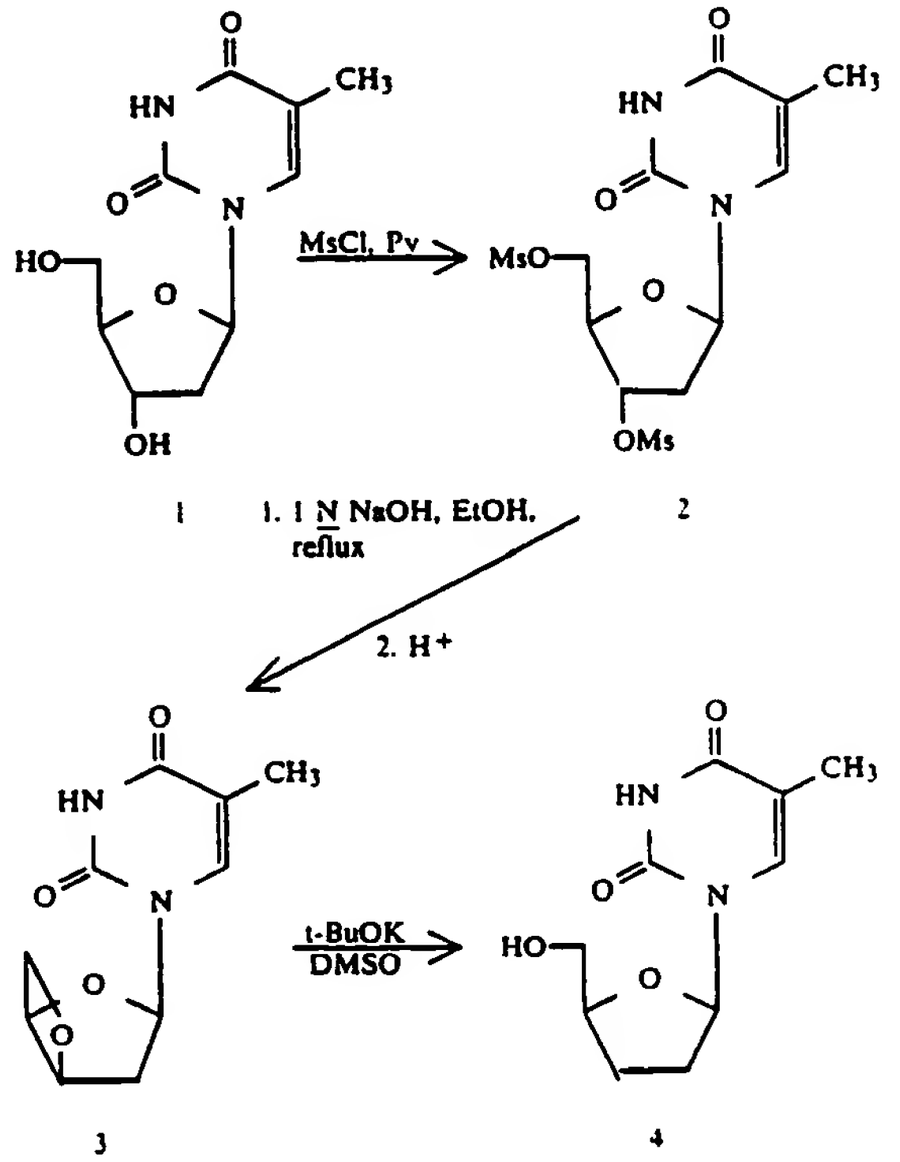


3'-Deoxythymidin-2'-ene (3'-deoxy-2', 3'-didehydrothymidine) has antiviral activity against retroviruses, e.g., murine leukemia virus and human immunodeficiency virus, i.e., HIV; HTLV III/LAV virus (the AIDS virus).

Retroviruses are RNA viruses whose genome contains copies of high-molecular weight single-stranded RNA. The virion contains reverse transcriptase. Nonlimiting examples of retroviruses include leukemia and

sarcoma viruses of animals, foamy viruses of primates and some slow viruses, e.g., visna and maedi of sheep.

A synthesis for the active compound of the present invention is illustrated in the following reaction scheme:



1 2 3 4

3,-Deoxythymidin-2-,ene (3,-deoxy-2',3,-didehydrothymidine) (4) can be synthesized basically by the methodology of J. P. Horwitz, J. Chua, M. A. DaRooge, M. Noel and I. L. Klundt, *J Org. Chem.*, 31, 205, (1966) with minor modifications. With reference to the above reaction scheme, treatment of thymidine (1) with methanesulfonyl chloride in pyridine at 0° C. gives the corresponding disulfonate 2. Refluxing compound,, 2 with 1 N NaOH solution in ethanol produces the 3',5'-cyclic ether 3. Treatment of compound 3 with potassium t-butoxide in dry DMSO yields the desired 2', 3'-unsaturated derivative 4.

The present invention provides a pharmaceutical composition containing as an active ingredient 3'-deoxythymidin-2'-ene (3'-deoxy-2', 3'-didehydrothyn'idine) in admixture with a solid, liquid or liquefied gaseous diluent.

The invention further provides a pharmaceutical composition containing as an active ingredient the 3'-deoxythumidin-2'-ene (3'-deoxy-2', 3'-didehydrothymidine) in the form of a sterile and/or physiologically isotonic aqueous solution

The invention also provides a medicament in dosage unit form comprising 3'-deoxythymidin-2'-ene (3'-deoxy-2', 3'-didehydrothymidine).

The invention also provides a medicament in the form of tablets (including lozenges and granules), caplets, dragees, capsules, pills, ampoules or suppositories comprising 3'-deoxythymidin-2'-ene (3'-deoxy-2', 3'-didehydrothymidine).

"Medicament" as used herein means physically discrete coherent portions suitable for medical administration. "Medicament in dosage unit form" as used herein means physically discrete coherent units suitable for

medical administration, each containing a daily dose or a multiple (up to four times) or a sub-multiple (down to a fortieth) of a daily dose of the compound of the invention in association with a carrier and/or enclosed within an envelope. Whether the medicament contains a daily dose, or for example, a half, a third or a quarter of a daily dose will depend on whether the medicament is to be administered once or, for example, twice, three times or four times a day, respectively.

The pharmaceutical compositions according to the invention may, for example, take the form of suspensions, solutions and emulsions of the active ingredient in aqueous or non-aqueous diluents, syrups, granulates or powders.

The diluents to be used in pharmaceutical compositions (e.g., granulates) adapted to be formed into tablets, dragees, capsules and pills include the following: (a) fillers and extenders, e.g., starch, sugars, mannitol and silicic acid; (b) binding agents, e.g., carboxymethyl cellulose and other cellulose derivatives, alginates, gelatine and polyvinyl pyrrolidone; (c) moisturizing agents, e.g., glycerol; (d) disintegrating agents, e.g., agaragar, calcium carbonate and sodium bicarbonate; (e) agents for retarding dissolution, e.g., paraffin; (f) resorption accelerators, e.g, quaternary ammonium compounds; (g) surface active agents, e.g., cetyl alcohol, glycerol monostearate; (h) adsorptive carriers, e.g., kaolin and bentonite; (i) lubricants, e.g., talc, calcium and magnesium stearate and solid polyethyl glycols.

The tablets, dragees, capsules, caplets and pills formed from the pharmaceutical compositions of the invention can have the customary coatings, envelopes and protective matrices, which may contain opacifiers. They can be so constituted that they release the active ingredient only or preferably in a particular part of the intestinal tract, possibly over a period of time. The coatings, envelopes and protective matrices may be made, for example, from polymeric substances or waxes.

The active ingredient can also be made up in microencapsulated form together, with one or several of the above-mentioned diluents.

The diluents to be used in pharmaceutical compositions adapted to be formed into suppositories can. for example. be tho usual water-soluble diluents, such as polyethylene glycols and fats (e.g., cocoa oil and high esters, [e.g., C_{14}-alcohol with C_{16}-fatty acid]) or mixtures of these diluents.

The pharmaceutical compositions which are solutions and emulsions can, for example, contain the customary diluents (with, of course, the above-mentioned exclusion of solvents having a molecular weight below 200, except in the presence of a surface-active agent), such as solvents, dissolving agents and emulsifiers. Specific non-limiting examples of such diluents are water, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (for example, ground nut oil), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitol or mixtures thereof.

For parenteral administration, solutions and emulsions should be sterile and, if appropriate, blood-isotonic.

The pharmaceutical compositions which are suspensions can contain the usual diluents, such as liquid diluents, e.g., water, ethyl alcohol, propylene glycol, sur-

face-active agents (e.g., ethoxylated isostearyl alcohols, polyoxyethylene sorbite and sorbitane esters), microcrystalline cellulose, aluminium metahydroxide, bentonite, agar-agar and tragacanth or mixtures thereof.

All the pharmaceutical compositions according to the invention can also contain coloring agents and preservatives, as well as perfumes and flavoring additions (e.g., peppermint oil and eucalyptus oil) and sweetening agents (e.g., saccharin and aspartame).

The pharmaceutical compositions according to the invention generally contain from 0.5 to 90% of the active ingredient (3'-deoxythymidin-2'-ene (3'-deoxy-2',3'-didehydrothymidine)) by weight of the total composition.

In addition to 3'-deoxythymidin-2'-ene (3'-deoxy-2',3'-didehydrothymidine), the pharmaceutical compositions and medicaments according to the invention can also contain other pharmaceutically active compounds.

Any diluent in the medicaments of the present invention may be any of those mentioned above in relation to the pharmaceutical compositions of the present invention. Such medicaments may include solvents of molecular weight less than 200 as the sole diluent.

The discrete coherent portions constituting the medicament according to the invention will generally be adapted by virtue of their shape or packaging for medical administration and may be, for example, any of the following: tablets (including lozenges and granulates), pills, dragees, capsules, suppositories and ampoules. Some of these forms may be made up for delayed release of the active ingredient. Some, such as capsules, may include a protective envelope which renders the portions of the medicament physically discrete and coherent.

The preferred daily dose for administration of the medicaments of the invention is 2.5 to 250 mg of active ingredient in the case of intravenous administration and 25 to 250 mg of active ingredient in the case of oral administration.

The production of the above-mentioned pharmaceutical compositions and medicaments is carried out by any method known in the art, for example, by mixing the active ingredient(s) with the diluent(,s) to form a pharmaceutical composition (e.g., a granulate) and then forming the composition into the medicament (e.g. tablets).

This invention provides a method for treating the above-mentioned diseases in warm-blooded animals, which comprises administering to the animals the compound of the invention, namely, 3'-deoxythymidin-2'-ene (3'-deoxy-2', 3'-didehydrothymidine), alone or in admixture with a diluent or in the form of a medicament according to the invention.

It is envisaged that this active compound, namely, 3'-deoxythymidin-2'-ene (3'deoxy-2', 3'-didehydrothymidine), will be administered perorally, parenterally (for example, intramuscularly, intraperitoneally, subcutaneously or intravenously), rectally or locally, preferably orally or parenterally, especially perlingually or intravenously. Preferred pharmaceutical compositions and medicaments are, therefore, those adapted for administration such as oral or parenteral administration. Administration in the method of the invention is preferably oral or parenteral administration.

In general, it has proved advantageous to administer intravenously amounts of from 0.01 mg to 10 mg/kg, preferably 0.05 to 5 mg/kg, of body weight per day and to administer orally 0.05 to 20 mg/kg, preferably 0.5 mg

to 5 mg/kg of body weight per day, to achieve effective results. Nevertheless, it can at times be necessary to deviate from those dosage rates, and in particular to do so as a function of the nature and body weight of the human or animal subject to be treated, the individual reaction of this subject to the treatment, type of formulation in which the active ingredient is administered, the mode in which the administration is carried out and the point in the progress of the disease or interval at which it is to be administered. Thus, it may in some case suffice to use less than the above-mentioned minimum dosage rate, whilst other cases the upper limit mentioned must be exceeded to achieve the desired results. Where larger amounts are administered, it may be advisable to divide these into several individual administrations over the course of the day.

The invention will now be described with reference to the following non-limiting examples.

EXAMPLE 1:

SYNTHESIS OF 3'-DEOXYTHYMIDIN-2'-ENE (3'-DEOXY-2', 3 -DIDEHYDROTHYMIDINE)

A solution of the cyclic ether 3 (see the reaction scheme described hereinabove) (8.64 g, 38.4 mmol) in 240 ml of dried DMSO containing 8.70 g (76.4 mmol) of potassium t-butoxide was stirred at room temperature for two hours. The reaction mixture was neutralized to a pH of approximately 7 with ethanolic acetic acid, and the solution was then evaporated to dryness at approximately 50° C. under reduced pressure. The residue was triturated with several portions of hot acetone. The insoluble materials were removed by filtration, and the filtrate was evaporated to dryness. The residue was eluted through a silica gel column ($CHCl_3$-EtOH, 2:1) to yield 6.5 g (76%) of product: mp 158°–160° C.; (Me_2SO-d_6) δ1.82 (s, 3H, 5-CH_3), 3.53 (m, 2H, 5'-H), 4.80 (m, 1H, 4'-H), 4.96 (t, 1H, 5'-OH, D_2O exchangeable), 5.90 (m, 1H, 3'-H, vinyl), 6.40 (m, 1H, 2'-H, vinyl), 6.82 (m, 1H, 1'-H), 7.67 (s, 1H, 6-H).

EXAMPLE 2:

BIOLOGICAL ASSAY PROCEDURE FOR ANTIVIRAL ACTIVITY AGAINST THE HUMAN IMMUNODEFICIENCY VIRUS (HIV; HTLV-III/LAV)

Three day-old mitogen stimulated human peripheral blood mononuclear (PBM) cells (10^6 per ml) were infected with HIV (strain LAV) in the presence and absence of various concentrations of 3-deoxythymidin-2'-ene, 1, 10, 100 μM. Five days after infection, the virus in the supernatant was pelleted and, after disruption, the reverse transcriptase activity was determined.

The methods used for culturing the PBM cells, harvesting the virus and determination of reverse transcriptase activity were those described by J. S. McDougal, S. P. Cort, M. S. Kennedy, C. D. Cabridilla, P. M. Feorino, D. P. Francis, D. Hicks, V. S. Kalyanaramen and L. S. Martin, *J. Immun. Meth.*, 76, 171, (1985). The virus was added to the cultures at the same time as the drug.

The data obtained indicated that essentially complete inhibition of the replication of the "AIDS" virus was obtained (>98% inhibition) at all three concentrations.

EXAMPLE 3:

BIOLOGICAL ASSAY PROCEDURE FOR ANTIVIRAL ACTIVITY AGAINST MOLONEY MURINE LEUKEMIA VIRUS (M-MuLV) BY XC-ASSAY

The XC assay system is an indirect method for quantitation of murine-leukemia virus (MuLV) originally described by V. Klement, W. P. Rowe, J. W. Hartley and W. E. Pugh, *Proc. Natl. Acad. Sci.*, 63, 753, (1969) and modified by W. P. Rowe, W. E. Pugh and J. W. Hartley, *Virology*, 42, 1136, (1970). This test is based on the development of syncytial changes in the XC cell line when it is co-cultivated with mouse fibroblast cells (SC-1 cells) productively infected with MuLV. The XC cell line was derived from a rat tumor induced by the prague strain of Rouse Sarcoma Virus (RSV) (J. Svoboda, P. Chyle, D. Simkovic and J. Hilgert, *Folia Biol.*, 9, 77, 1963)). This cell line contains the RSV genome, but does not produce infectious virus in the absence of a helper virus.

10E6 SC-1 cells were seeded in Earls Minimum Essential Medium (EMEM)-10% Fetal Bovine Serum (FBS), onto 60 mm petri dishes. The following day, the cells were inoculated with 0.5 ml of a virus dilution containing 25 μg/ml of DEAE-dextran. The dishes were maintained for 1 hour at 37° C. in a humidified 5% CO_2 incubator. The virus inoculum was then removed and replaced with 5 ml of medium containing appropriate concentrations of the test compound (two dishes/concentration). Medium containing 10% FBS was added to the virus control dishes. The medium (with or without the test compound) was changed at 48 hours.

Five days after virus inoculation, the culture fluid was decanted, and the cells were irradiated with a "General Electric" germicidal bulb for 30 seconds (1500–1800 ergs UV-light). Cultures were immediately overlaid with 10E6 SC cells in 5 ml of EMEM-10% FBS/dish. The medium was changed at 2-day intervals. Four days after XC cells addition, cultures were simultaneously fixed and stained with GEIMSA for 10 to 15 minutes.

Plaques were counted using an inverted microscopy as holes in the cell sheet containing syncytial cells, or as focal masses of multinucleated giant cells. The antiviral

activity was highly significant and had an ED_{50} of 2.5 μM.

Calculation of % Inhibition/Concentration (% Inh./conc.):

$$\%\ \text{Inh./conc.} = 100 - \left[\frac{\text{average \# of syncytial/conc. of test compound}}{\text{average \# of syncytia/in the virus control}} \times 100\right]$$

ED_{50}: Accumulative % Inhibition using the Reed-Muench Method

It will be appreciated that the instant specification and claims are set forth by way of illustration and not limitation and that various modifications and changes may be made without departing from the spirit and scope of the present invention.

What is claimed is:

1. A method for treating warm blooded animals infected with a retrovirus, the method comprising administering to the warm blooded animal an anti-retroviral effective amount of 3'-deoxythymidin-2'-ene, either alone or in admixture with a diluent or in the form of a medicament.

2. A method according to claim 1, wherein the retrovirus is Moloney murine leukemia virus.

3. A method according to claim 1, wherein the retrovirus is HTLV III/LAV.

4. A method according to claim 1, wherein the 3'-deoxythymidine-2'-ene is administered intravenously in an amount of 0.01 to 10 mg per kg body weight per day.

5. A method according to claim 1 wherein the 3'-deoxythymidin-2'-ene is in admixture with a solid, liquid or liquified gaseous diluent to form a pharmaceutical composition.

6. A method according to claim 5, wherein the pharmaceutical composition contains 0.5 to 90% of said 3'-deoxythymidin-2'-ene.

7. A method according to claim 5, wherein the pharmaceutical composition is in the form of a sterile physiologically isotonic aqueous solution.

8. A method for treating human blood cells infected with HIV comprising administering to said cells an antiretroviral effective amount of 3'-deoxythymidin-2'-ene either alone or in admixture with a diluent or in the form of a medicament.

* * * * *

EXHIBIT 3

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 4,978,655

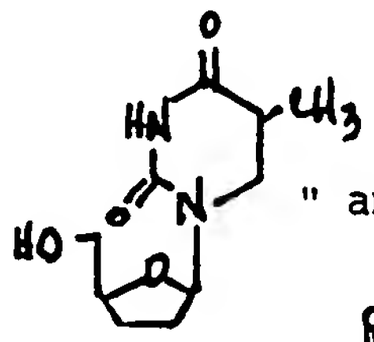
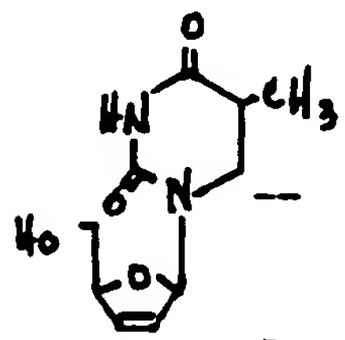
DATED : December 18, 1990

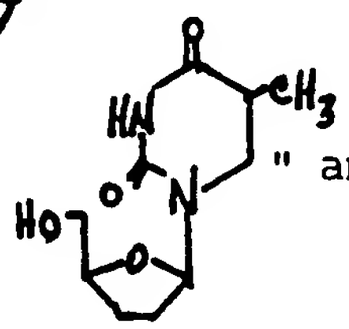
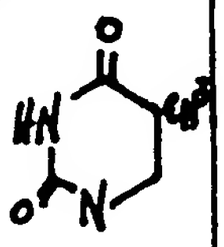
INVENTOR(S) : Lin et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title Page — [54] TITLE: 2nd line after 1st 3' " insert -- - --

Col. 1, line 2 — Second line of Title after 1st " 3' " insert -- - --

Col. 2, lines 50- — Delete "  " and substitute --  --

Col. 3, lines 23- — No. 4 delete "  " and substitute -- 

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 4,978,655

DATED : December 18, 1990

INVENTOR(S) : Lin, et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Col. 3, line 49 Delete " didehydrothymidine " and substitute -- didehydrothymidine --

Signed and Sealed this

Twenty-seventh Day of October, 1992

Attest:

DOUGLAS B. COMER

Attesting Officer

Acting Commissioner of Patents and Trademarks